KAMIYA BIOMEDICAL COMPANY

Cathepsin B Detection Kit

For the detection of active Cathepsin B in living cells without lysis using a Magic Red-labeled substrate of Cathepsin B (MR-$(RR)_2$).

Cat. No. KT-088 (100 tests/kit)

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Cathepsin B Detection Kit can be used to detect active Cathepsin B in living cells using fluorescence microscopy.

DESCRIPTION

Elevated cathepsin enzyme activity in serum or the extracellular matrix often signifies a number of gross pathological conditions. Cathepsin-mediated diseases include: Alzheimer's; numerous types of cancer; autoimmune related diseases like arthritis; and the accelerated breakdown of bone structure seen with osteoporosis. Up-regulated cathepsin B and L activity has been linked to several types of cancer. These include cancer of the colon, pancreas, ovaries, breast, lung, and skin (melanoma). Up-regulation of cathepsin K has been shown in lung tumors. Increased cathepsin K activity has also been linked to degenerative bone diseases including osteopetrosis and post-menopausal osteoporosis.

Cathepsins are usually characterized as members of the lysosomal cysteine protease (active site) family and the cathepsin family name has been synomonous with lysosomal proteolytic enzymes. In actuality, the cathepsin family also contains members of the serine protease (cathepsin A,G) and aspartic protease (cathepsin D,E) families as well. These enzymes exist in their processed form as disulfide-linked heavy and light chain subunits with molecular weights ranging from 20-35 kDa. Cathepsin C is the noted exception, existing as an oligomeric enzyme with a MW ~200 kDa. Initially synthesized as inactive zymogens, they are post-translationally processed into their active configurations after passing through the endoplasmic reticulum and subsequent incorporation into the acidic environment of the lysosomes.

Cathepsin activity can be detected within whole living cells using Magic Red™ substrate-based **K-ASSAY®** Cathepsin Detection Kits. These kits allow researchers to quickly visualize intracellular cathepsin activity within their particular experimental cell line.

PRINCIPLE

The **K-ASSAY®** Magic Red™ (MR) Cathepsin Detection kits utilize the fluorophore, cresyl violet. When bi-substituted via amide linkage to two cathepsin target sequence peptides {such as $(Arginine\text{-}Arginine)_2$}, the cresyl violet leaving group is non-fluorescent. Following enzymatic cleavage at one or both arginine (R) amide linkage sites, the mono and non-substituted cresyl violet fluorophores generate red fluorescence when excited at 550-590 nm.

KAMIYA BIOMEDICAL COMPANY has taken the cresyl violet fluorophore derivatives of $(z\text{-}Arginine\text{-}Arginine)_2$ abbreviated as $(z\text{-}RR)_2$, $(z\text{-}Leucine\text{-}Arginine)_2$ abbreviated as $(z\text{-}LR)_2$, and $(z\text{-}Phenylalanine\text{-}Arginine)_2$ abbreviated as $(z\text{-}FR)_2$ and incorporated them into user-friendly assay kits. These MR-Cathepsin photostable fluorogenic substrates easily penetrate the cell membrane and the membranes of the internal cellular organelles enabling researchers to detect cathepsin activity within whole living cells. Intracellular cresyl violet substrate hydrolysis can be monitored by the accumulation of red fluorescent product within various organelles. By varying the duration and concentration of exposure to the MR-Cathepsin substrate, a picture can be obtained of the relative abundance and intracellular location of cathepsin enzyme activity. This activity can be monitored using a fluroescence microscope or microtiter plate fluorometer.

The unsubstituted red fluorescent MR-Cathepsin product has an optimal excitation and emission wavelength pairing of 592 nm and 628 nm respectively. At these higher excitation wavelengths, the amount of cell-mediated auto-fluorescence is minimal. Fortunately, the excitation peak of this fluorophore is rather broad allowing good excitation efficiency at 540-560 nm. The typical mercury lamp used in fluorescence microscopy has a maximum light output at 542 nm which is quite compatible with the Magic Red™ substrate.

Hoechst stain is included and can be used to label the cell nuclei after labeling with the MR-Cathepsin reagent. It is revealed under a microscope using a UV-filter with excitation at 365 nm and emission at 480 nm.

In addition, acridine orange (AO) is included in the kit to help identify lysosomes and other intracellular organelles. The acidic pH of the lysosome results in the concentration and aggregation of the AO molecules. Aggregated AO molecules fluoresce orange rather than green thus clearly differentiating the lysosomes from the other organelles (see figure 3).

COMPONENTS

- 1 vial MR-$(RR)_2$ reagent, lyophilized (reconstitute in 200 uL)
- 1 vial (0.5 mL) AO
- 1 vial (1 mL) Hoechst Stain

Materials or equipment required but not provided

- Cultured cells with media
- Reagents to induce experimental condition
- 15 mL polystyrene centrifuge tube (1 per sample)
- Amber vials or polypropylene tubes for storage of 260X concentrate at –20°C, if aliquoted
- Slides and coverslips
- Hemocytometer
- Clinical centrifuge at <200 X g
- 37°C CO_2 incubator
- Pipette(s) capable of dispensing at 10µL, 50µL, 200µL, 300µL, 1mL
- Deionized (DI) H_2O, up to 2 mL
- Phosphate Buffered Saline (PBS) pH 7.4, up to 100 mL
- Dimethylsulfoxide (DMSO), 50µL (25 tests/kit) or 200µL (100 tests/kit)
- Trypsin versine
- Ice or 4°C refrigerator to store cells
- Instrumentation
    - Fluorescence microscope with appropriate filters (excitation 550 nm, emission >610 nm for MR-$(RR)_2$; excitation at 480 nm and emission at >540 nm for AO; and if Hoechst is used, a UV-filter with excitation at 365 nm and emission at 480 nm).

PROTOCOLS

PREPARATION OF REAGENTS

Hoechst 33342 Stain

Hoechst stain can be used to label the cell nuclei after labeling with the MR-$(RR)_2$ reagent. It can be observed under a microscope equipped with a UV-filter with excitation at 365 nm and an emission at 480 nm. Hoechst stain is provided ready-to-use at 200 µg/mL.

Warning: Hoechst stain is a potential mutagen. Use of gloves, protective clothing and eyewear are strongly recommended. When disposing, flush sink with copious amounts of water.

Acridine Orange

Acridine orange (AO) is a chelating dye and can be used to reveal lysosomes, nuclei, and nucleoli. 0.5 mL of AO is provided at 1 mM. AO may be used neat or diluted in diH_2O or media prior to pipetting into the cell suspension. Always protect AO from bright light.

Lysosomal structures can be visualized using AO concentrations ranging from 0.5 to 5.0 µM. This concentration range can be obtained by diluting the AO reagent stock 1:2000 to 1:200 (0.05 – 0.5% v/v) into the final cell suspension. For example, if using AO at 1.0 µM in the final cell suspension, first dilute the AO 1:100 in diH_2O: put 10 µL AO into 990 µL diH_2O. Then pipette the AO into the cell suspension at 1:10: put 55.5 µL diluted AO into 500 µL cell suspension.

As AO exhibits a very broad emission range, one of several filter pairings on the fluorescence microscope can be used to view this stain. The same excitation/emission pairing filters used to view the MR-$(RR)_2$ can also be used: a 550 nm (540-560 nm) excitation and long pass >610 nm emission/barrier filter pairing. In this case, the lysosomes appear red instead of yellowish green.

When illuminating with a blue light (480 nm) excitation filter, a green light (540-550 nm) emission/barrier filter combination works well. Lysosomes will appear yellowish green. As this filter combination is very close to the maximum emission of AO, the slide may appear too bright. Cells read at this combination may need to be washed prior to viewing to remove any excess AO.

Because of the emissions overlap, dual staining of cells with both MR-$(RR)_2$ and AO will yield confusing results. Therefore these dyes should not be used to stain the same cells.

Warning: AO is a potent mutagen and probable carcinogen. Use of gloves, protective clothing, and eyewear are strongly recommended. When disposing, flush sink with copious amounts of water.

Reconstitution of the 260X MR-$(RR)_2$ Stock

The MR-$(RR)_2$ reagent is a highly concentrated lyophilized powder. It must first be reconstituted in DMSO, forming a 260X stock concentrate, and then diluted 1:10 in PBS to form a final 26X working solution. For best results, the 26X working solution should be prepared immediately prior to use; however, the reconstituted 260X stock concentrate can be stored at or below –20°C for future use.

The newly reconstituted 260X MR-$(RR)_2$ stock must be used or frozen immediately after it is prepared. Protect the MR-$(RR)_2$ stock from light during handling.

1. Reconstitute the lyophilized MR-$(RR)_2$ with 200 µL of DMSO to yield a 260X concentrate.
2. Mix by swirling or tilting the vial, allowing the DMSO to travel around the base of the vial until completely dissolved. At room temperature (RT), the reagent should be dissolved within a few minutes.
3. If immediately using this solution, dilute it to 26X.
4. Or, if using later, aliquot and store it at or below –20°C.

Preparation of 26X MR-$(RR)_2$ Solution for Immediate Use

Using the freshly reconstituted 260X MR-$(RR)_2$ stock, prepare the 26X working-strength MR-$(RR)_2$ solution by diluting the stock 1:10 in PBS. Following the suggested protocols here, each 0.5 mL sample to be tested requires only 20 µL of 26X MR-$(RR)_2$ solution (or 2 µL of the 260X MR-$(RR)_2$ stock).

1. If using the entire vial, add 1.8 mL PBS (the vial contains 200 µL of the 260X stock; this yields 2 mL of a 26X solution).
2. If not using the entire vial, dilute the 260X stock 1:10 in PBS. For example, add 10 µL of the 260X stock to 90 µL PBS (this yields 100 µL of a 26X solution). Store the unused 260X stock at or below –20°C.
3. Mix by inverting or vortexing the vial at RT.

The 26X working strength MR-$(RR)_2$ solution must be used the same day that it is prepared.

Storage of 260X MR-$(RR)_2$ Stock for Future Use

If not all of the 260X MR-$(RR)_2$ stock will be used the same time it is reconstituted, the unused portion may be stored at or below -20°C for 6 months. During that time, the 260X MR-$(RR)_2$ stock may be thawed and used twice. After the second thaw, discard any remaining 260X MR-$(RR)_2$ stock. If you anticipate using it more than twice, make small aliquots in amber vials or polypropylene tubes and store at or below -20°C protected from light.

Preparation of 26X MR-$(RR)_2$ Solution from a Frozen Aliquot

If some of the 260X MR-$(RR)_2$ reagent was previously reconstituted and then stored at or below -20°C, it may be used 2 more times within 6 months.

1. Thaw the 260X MR-$(RR)_2$ stock and protect from light.
2. Once the aliquot has become liquid, dilute the 260X stock solution 1:10 in PBS and vortex. For example, mix 10 µL of 260X MR-$(RR)_2$ reagent with 90 µL PBS.
3. If the 260X MR-$(RR)_2$ stock was frozen immediately after reconstitution and was never thawed, return it to the freezer. If the stock was thawed once before, discard it.

4. Proceed to the labeling protocol.

PROCEDURE

Overview of the Protocol

Staining cells with the MR-Cathepsin kit can be completed within a few hours. However, the MR-Cathepsin kit is used with living cells, which require periodic maintenance and cultivation several days in advance. Therefore, as the 26X MR-$(RR)_2$ solution must be used immediately, the MR-$(RR)_2$ reagents should not be prepared until just prior to staining. The following is a quick overview of the MR-Cathepsin protocol:

1. Culture cells to the density optimal for your experimental condition, but not to exceed 10^6 cells/mL.
2. At the same time, culture a non-induced negative control cell population at the same density as the induced population for every labeling condition. For example, if labeling with MR-$(RR)_2$, Hoechst stain and AO, make 5 populations:

   a. Unlabeled cells.
   b. MR-$(RR)_2$-labeled cells.
   c. MR-$(RR)_2$- and Hoechst-labeled cells.
   d. Hoechst-labeled cells.
   e. AO-labeled cells.

3. Reconstitute the vial of lyophilized MR-$(RR)_2$ with DMSO to form the 260X MR-$(RR)_2$ stock concentrate.
4. Dilute the 260X MR-$(RR)_2$ stock to the 26X working solution.
5. Stain cells by adding the 26X MR-$(RR)_2$ solution.
6. Incubate cells for ~1 hour.
7. If necessary, wash and spin cells.
8. If desired, label cells with Hoechst stain.
9. If desired, label cells that have not been exposed to MR-$(RR)_2$ with AO.
10. Analyze data via fluorescence microscopy.

Fluorescence Microscopy Staining Protocol for Suspension Cells

1. Culture cells to a density optimal for the cell line being studied.
2. Cultivate or concentrate cells to a density of at least 5 X 10^5 cells/mL. Cells may be concentrated by centrifugation just prior to staining.

   Cell density in the cell culture flasks should not exceed 10^6 cells/mL. Cells cultivated in excess of this concentration may begin to naturally enter apoptosis due to nutrient deprivation or the accumulation of cell degradation products in the media. Optimal cell concentration will vary depending on the cell line used.

3. Expose cells to the condition being investigated.
4. At the same time, culture an equal volume of negative control cells for use as a reference population. Make sure that both the experimental (positive) cell population tubes and the negative control tubes contain similar quantities of cells.

   When ready to label with the 26X MR-$(RR)_2$ solution, cells should be between 5 X 10^5 – 2 X 10^6 cells/mL for best viewing.

5. If necessary, concentrate cells by gentle centrifugation at 200 X g for 3 –8 minutes.
6. Prepare the 260X stock by reconstituting the vial of lyophilized MR-$(RR)_2$ with 200 µL (100-test vials) DMSO.
7. Prepare the 26X stock by diluting the reconstituted vial with1.8 mL (100-test vials) PBS.
8. Transfer 500 µL of each induced and negative control cell populations into fresh 12 x 75 mm glass or polypropylene tubes. Or, if desired, larger cell volumes can be used, however more of the 26X MR-$(RR)_2$ solution may be required. Larger volume cell suspensions label nicely using 25 cm^2 tissue culture flasks (laid flat) as incubator vessels.
9. Add 20 µL of the 26X working dilution MR-$(RR)_2$ solution directly to each 500 µL cell suspension forming a final volume of 520 µL.
10. Or, if a different volume was used, add the 26X MR-$(RR)_2$ solution at a 1:26 ratio of the final volume. For example, if 1,000 µL of cell suspension was used, add 40 µL of the 26X MR-$(RR)_2$ solution forming a final volume of 1,040 µL.

(To optimize this assay to your specific research conditions, adjust the amount of 26X MR-$(RR)_2$ used to determine the greatest difference in the fluorescence signal between positive and negative cell populations.)

Each investigator should titrate the amount of MR-$(RR)_2$ used to accommodate their particular cell line and research conditions.

11. Mix the cells thoroughly.
12. Incubate cells for 15 to 60 minutes at 37°C under 5% CO_2, protecting the tubes from light. As cells may settle on the bottom of the tubes, gently resuspend them by swirling the tubes every 20 minutes during this incubation time. This will ensure an even distribution of the MR-$(RR)_2$ reagent among all cells. (To optimize this assay to your specific research conditions, adjust this incubation time to determine the greatest difference in the fluorescence signal between positive and negative cell populations.)

Each investigator should adjust the incubation time to accommodate their particular cell line and research conditions.

13. At this point, the cells may be stained with Hoechst stain, and cells unexposed to MR-$(RR)_2$ can be stained with AO.
14. If cells are to be monitored using Hoechst stain:
    a. Add 2.5 µL Hoechst stain (0.5% v/v) to the 520 µL cell suspension labeled with MR-$(RR)_2$.
    b. Or, add 2.5 µL Hoechst stain (0.5% v/v) to a 500 µL cell suspension that was not labeled.
    c. Incubate for an additional 5 – 10 minutes at 37°C under 5% CO_2.
    d. Go to Step 16.
15. If cells are to be monitored using AO instead of MR-$(RR)_2$:
    a. Pipette the AO reagent stock 1:2000 to 1:200 (0.05 – 0.5% v/v) into the final cell suspension. Because of the emissions overlap, dual staining of cells with both MR-$(RR)_2$ and AO will yield confusing results. Therefore, the dyes should be used separately.
    For example, if using AO at 1.0 µM in the final cell suspension, first dilute the AO 1:100 in diH_2O: put 10 µL AO into 990 µL diH_2O. Then pipette the diluted AO into the cell suspension at 1:10: put 55.5 µL diluted AO into 500 µL cell suspension.
    b. Incubate for an additional 30 minutes at 37°C under 5% CO_2.
    c. If viewing under the same filters used for the MR-$(RR)_2$ staining (excitation at 550 nm, emission >610 nm), cells may be viewed immediately after staining, without a wash step - go to Step 16.
    d. If viewing under blue (480 nm) excitation and green (540-550 nm) emission wavelengths, cells may need to be washed with PBS to remove any excess AO as the cells may appear too bright. Brightness will depend on the type of microscope used, and the type of cell line. To wash the cells:
        i) Gently pellet cells at 200 X g for 3-8 minutes at RT.
        ii) Remove and discard supernatant.
        iii) Resuspend cells in a similar volume of PBS.
        iv) Go to Step 16.
16. Place 15 – 20 µL of the cell suspension onto a microscope slide and cover with a coverslip.
17. Observe MR-$(RR)_2$-stained cells using a fluorescence microscope equipped with an excitation filter of 550 nm (540 – 560 nm) and a long pass >610 nm emission/barrier filter pairing. (If these filters are not available, select a filter combination that best approximates these settings.) Using this excitation/emission filter pairing, cells should stain red with more brightly stained vacuoles and lysosomes.
18. If the same sample was stained with both MR-$(RR)_2$ and Hoechst, and if a multi-wavelength filter option is available on the fluorescence microscope, the dual staining properties of the sample can be examined. Hoechst stain can be seen using a UV-filter with excitation at 365 nm and emission at 480 nm. If these exact filter pairings are not available, select a filter combination that best approximates these settings.
19. As AO exhibits a very broad emission range, one of several filter pairings may be used. The same excitation/emission pairing filters used to view the MR-$(RR)_2$ may be used: a 550 nm (540-560 nm) excitation and long pass >610 nm emission/barrier filter pairing. In this case, the lysosomes appear red. When illuminating with a blue light (480 nm) excitation filter, a green light (540-550 nm) emission/barrier filter combination works well. Lysosomes will appear yellowish green instead of red. Because of the emissions overlap, dual staining of cells with both MR-$(RR)_2$ and AO will yield confusing results. Therefore, these dyes should be used separately.
20. To optimize this assay for your specific research conditions, vary the amount of 26X MR-$(RR)_2$ used (Steps 9, 10), and the incubation time (Step 12) to determine the greatest difference in the fluorescence signal between induced and non-induced cell populations.

Fluorescence Microscopy Staining Protocol for Adherent Cells

1. Select the cell culture flask containing the cells that will be studied and dislodge the attached cells.

a. Aseptically remove the media from the flask.
b. Depending on the size of your culture flask, aseptically add 5 - 25 mL sterile PBS or saline to the flask.
c. Aseptically remove this solution and discard.
d. Add trypsin-versine to the flask, varying the amount depending on the flask surface area. For example, a 25 cm^2 flask should receive approximately 1 mL of the trypsin reagent; a 75 cm^2 flask should receive approximately 3 mL.
e. Incubate the flask for 1-2 minutes, rocking the flask gently back and forth to dislodge the attached cells.
f. To neutralize the trypsin activity and count the cells, dilute the contents of the flask 1:20 into culture media. For example, take 1 mL of suspension and add to a sterile culture tube containing 19 mL of the complete cell culture media (with serum)
g. Count the cells using a hemocytometer.

2. Seed about 10^4 – 10^5 cells onto a sterile coverslip in a 35 mm petri dish or onto chamber slides.
3. Grow the cells using your culture media formulation until about 80% confluent. This usually takes about 24 hours, but will vary with your cell line.
4. Expose cells to your experimental conditions.
5. Remove samples of the cell overlay media at time points according to your specific protocol. The precise volume used should be optimized to your specific conditions. For example, if working in a microtiter plate, 300 µL samples may be appropriate, or if working in a chamber slide, 1 mL or more of the sample may be necessary.
6. Add the 26X MR-$(RR)_2$ solution directly to each sample at a ratio of 1:26. For example, if 300 µL was used, add 12 µL of the 26X MR-$(RR)_2$ solution forming a final volume of 312 µL. If 1,000 µL was used, add 40 µL of the the 26X MR-$(RR)_2$ solution forming a final volume of 1,040 µL.
7. Optimize this assay to your specific research conditions by adjusting the volume of sample used and amount of 26X MR-$(RR)_2$ used to determine the greatest difference in the fluorescence signal between positive and negative cell populations.

Each investigator should titrate the amount of MR-$(RR)_2$ used to accommodate their particular cell line and research conditions.

8. Gently mix the cell overlay media to ensure an even exposure to the MR-$(RR)_2$.
9. Incubate cells for 30 - 60 minutes at 37°C in a CO_2 incubator.
10. Remove the media.
11. Rinse twice with PBS, 1 minute per rinse.
12. At this point, labeled and unlabeled cells can be stained with Hoechst stain, and unlabeled cells can be stained with AO.
13. If cells are to be monitored using Hoechst stain:
    a. Add 5.2 µL Hoechst stain (0.5% v/v) to the 1040 µL of cell overlay material labeled with MR-$(RR)_2$.
    b. Or, add 5.2 µL Hoechst stain (0.5% v/v) to 1040 µL cell suspension that was not labeled.
    c. Incubate for an additional 5 – 10 minutes at 37°C under 5% CO_2.
    d. Go to Step 15.
14. If cells are to be monitored using AO:
    a. Pipette the AO reagent stock 1:2000 to 1:200 (0.05 – 0.5% v/v) into the final cell overlay media. Because of the emissions overlap, dual staining of cells with both MR-$(RR)_2$ and AO will yield confusing results. Therefore, the dyes should be used separately.
    b. For example, if using AO at 1.0 µM in the final cell media, first dilute the AO 1:100 in PBS: put 10 µL AO into 990 µL PBS. Then pipette the diluted AO into the cell overlay media at 1:10: put 111 µL diluted AO into 1000 µL cell overlay media forming a final volume of 1111 µL.
    c. Incubate for an additional 30 minutes at 37°C under 5% CO_2.
    d. Remove the media from the cell mono-layer surface.
    e. Rinse twice with PBS, 1 minute per rinse.
    f. Go to Steps 15 and 18.
15. Mount the coverslip with cells facing down onto a drop of PBS. If a chamberslide was used, pull off the plastic frame and add a drop of PBS to the cell surface and cover with a coverslip.
16. Observe MR-$(RR)_2$ stained cells using a fluorescence microscope equipped with an excitation filter of 550 nm (540 – 560 nm) and a long pass >610 nm emission/barrier filter pairing. (If these filters are not available, select a filter combination that best approximates these settings.) Using this excitation/emission filter pairing, cells should stain red with more brightly stained vacuoles and lysosomes.
17. If the same sample was stained with both MR-$(RR)_2$ and Hoechst, and a multi-wavelength filter option is available on the fluorescence microscope, the dual staining properties of the sample can be examined. Hoechst stain can be seen using a UV-filter with excitation at 365 nm and emission at 480 nm. If these exact filter pairings are not available, select a filter combination that best approximates these settings.
18. As AO exhibits a very broad emission range, one of several filter pairings on the fluorescence microscope may be used. The same excitation/emission pairing filters used to view the MR-$(RR)_2$ may also be used: a 550 nm (540-560

nm) excitation and long pass >610 nm emission/barrier filter pairing. Lysosomes will appear red. When illuminating with a blue light (480 nm) excitation filter, a green light (540-550 nm) emission/barrier filter combination works well. In this case, lysosomes will appear yellowish green instead of red. Because of the emissions overlap, dual staining of cells with both MR-$(RR)_2$ and AO will yield confusing results. Therefore, these dyes should be used separately.

19. To optimize this assay for your specific research conditions, vary the amount of 26X MR-$(RR)_2$ used (Steps 6-7), and the incubation time (Step 9) to determine the greatest difference in the fluorescence signal between positive and negative cell populations.

Fluorescence Microscopy Sample Data

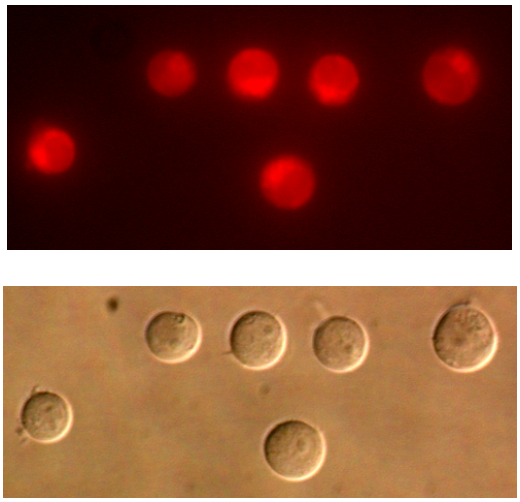

Figure 1. Detection of intracellular cathepsin B activity in Jurkat cells using (z-RR)$_2$-MR-Cathepsin fluorogenic substrate. Intracellular localization of the hydrolyzed (fluorescent) Magic Red™ product was detected on a Nikon Eclipse E800 photomicroscope using a 510 – 560 nm excitation filter and a 570 – 620 nm emission/barrier filter at 500X. Bottom photo shows the corresponding DIC image of the cells.

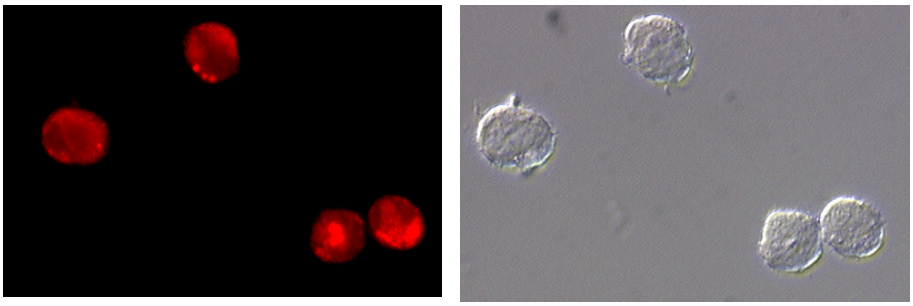

Figure 2. Detection of intracellular cathepsin B activity in THP-1 cells using (z-RR)$_2$-MR-Cathepsin fluorogenic substrate. Intracellular localization of the hydrolyzed (fluorescent) Magic Red™ product was detected on a Nikon Eclipse E800 photomicroscope using a 510 – 560 nm excitation filter and a 570 – 620 nm emission/barrier filter at 400X. Right photo shows the corresponding DIC image of the cells.

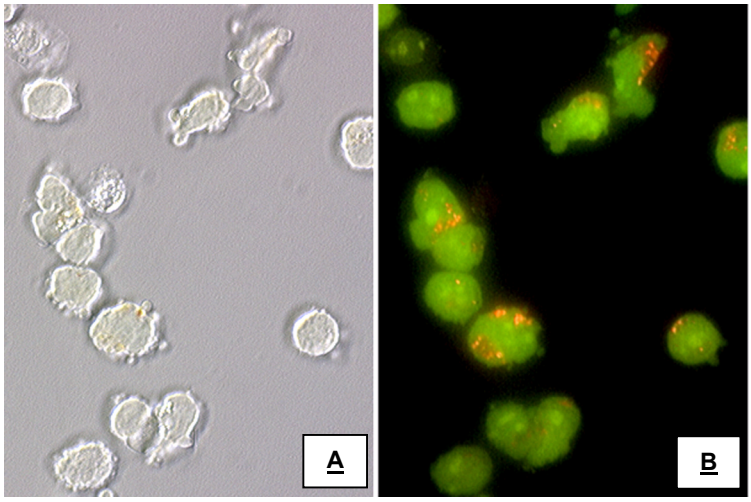

Figure 3. Acridine orange staining of normal Jurkat cells showing orange lysosomal staining. Jurkat cells were stained with 5 μM AO in PBS for 60 minutes at 37°C. Photomicrographs were taken using a Nikon Eclipse E800 photomicroscope using a 460-500nm excitation filter and a 505-560 nm emission / barrier filter set at 300X. Photo A shows the corresponding DIC image of the cells (AO appears faintly).

STORAGE

- Store the unopened kit (and each unopened components) at 4°C until the expiration date.
- Protect the probe reagent from light at all times.
- Once reconstituted, the 260X MR-$(RR)_2$ reagent stock should be stored at -20°C protected from light. This reagent is stable for up to 6 months and may be thawed twice during that time.

Warnings and Precautions

- Use gloves while handling the probe reagent, Hoechst stain and AO.
- Dispose of all liquid components down the sink and flush with copious amounts of water. Solid components may be tossed in standard trash bins.

FOR RESEARCH USE ONLY

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